EFFECT OF SALTS UPON OXIDASE ACTIVITY OF APPLE BARK

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 263

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(WITH FIVE FIGURES)

Introduction

In an earlier paper (21) one of the authors showed that there is a marked difference in the action of the salts of the alkali metals upon the fire-holding capacity of tobacco, even when the salts have similar anions. For instance, the carbonates of potassium, rubidium, and caesium promote the combustion of tobacco to a very much greater extent than the carbonates of sodium and lithium. The chlorides of sodium, lithium, and potassium retard the combustion, but the chloride of potassium is not nearly so effective as the chloride of sodium or lithium. In general, the salts of potassium, rubidium, and caesium are much more favorable to combustion than those of sodium and lithium.

It has been known for a long time that potassium is an essential element for the higher plants. Numerous attempts have been made to replace potassium by sodium, and, while apparently sodium can fulfil some of the functions of potassium, attempts to replace potassium entirely by sodium have been unsuccessful. The fact that potassium seems to have such a marked property of promoting the combustion of tobacco, and sodium does not, suggests that this particular property of potassium may have a relation to certain functions in the plant, which cannot be fulfilled by sodium. These facts suggested that a study of the effect of the alkali salts upon oxidase activity might be of interest. The work reported in this paper was done in 1917. More extended studies were planned, but, since it has been impossible to carry them out completely at the present time, it seemed wise to report the results obtained.

Historical

BERTRAND (5) was the first investigator to point out that the salts of metals influence oxidase activity. He showed that manganese salts greatly increase the oxidase activity of preparations from alfalfa. GESSARD (15) found that the formation of melanin from tyrosin is increased in the presence of salts of the metals. BACH (4) substantiated GESSARD'S results, and showed that aluminum sulphate, salts of calcium, magnesium, manganese, and zinc increase melanin formation from tyrosin. The effect of the salts is to increase the further change of the oxidation product rather than to activate the taking up of oxygen. Aluminum salts hasten the formation of purpurogallin from the yellow oxidation product of the action of oxidase upon pyrogallol. BACH believed that the oxidation process is retarded by the accumulation of the primary oxidation products, and that the salts act to release them. WOLFF (32) found that the oxidation of tyrosin by tyrosinase from *Russula delica* is increased by the addition of small quantities of disodiumphosphate. PORODKO (26), ASO (3), ALSBERG (2), and EWART (11) have shown that salts of the metals give a blue color with guaiacum. PORODKO and EWART believed these salts to be inorganic oxidases. PORODKO pointed out that those metals which form salts of two degrees of oxidation are particularly active. ALSBERG, and also EWART, confirmed PORODKO'S observation and found that the chlorides of many of the metals give a blue color with guaiacum. ALSBERG attributed an important part in the reaction to the chlorine. EWART further found that the chlorides, nitrates, and sulphates of the same metal are not necessarily equally powerful in their action. Apparently the chlorides are more active than the sulphates. Various salts were found to act as sensitizers or retardants to oxidase activity. Potassium chloride, potassium iodide, potassium bromide, and potassium fluoride retard or even prevent the browning of pounded apple pulp.

Numerous investigators have shown that oxidase activity is affected by changes in reaction of the medium. BERTRAND (6) showed that the action upon guaiacol of laccase from *Rhus succedanea* is inhibited by 0.002 M concentration of sulphuric acid.

WOLFF found tyrosinase from *Russula delica* most active in a solution neutral to phenolphthalein, and ABDERHALDEN and GUGGENHEIM (1) found that tyrosinase is destroyed by 0.016 N hydrochloric acid, and greatly retarded by 0.016 N sodium hydroxide. ROSE (28) showed that the decrease in oxidase activity, as observed in the Bunzell apparatus, is due to an increase in the hydrogen ion concentration of the medium. REED (27) found oxidase activity in potatoes and apples inhibited even by low hydrogen ion concentrations; and likewise BUNZELL (9) found the action of oxidase retarded with increasing hydrogen ion concentrations.

Methods

All but one of the experiments described in this paper were made with portions of apple bark which had been dried at 35–40° C. for 2–3 hours, ground fine enough to pass through a 40-mesh wire sieve, and stored air dry in zinc-capped Mason jars. One experiment was made with solutions of precipitated oxidase separated from aqueous extracts of healthy bark and of diseased bark by the addition of about 10 volumes of alcohol. In order to obtain the precipitated oxidase, 2 gm. of bark were allowed to stand in a beaker with 10 cc. of water and 5 drops of toluol for 1 hour. The extract was then squeezed out through moist cheesecloth on coarse filter paper. The beaker was washed with five 1 cc. portions of water and the filter paper finally with two more. There was then added 50 cc. of 95 per cent alcohol to the filtrate (concentration of alcohol about 70 per cent) and the whole allowed to stand for 10 minutes. The flocculent precipitate which had formed was collected on a hard filter by gentle suction with a filter pump. There was then added 150 cc. more alcohol to the filtrate (concentration of alcohol now about 90 per cent) and the whole allowed to stand for 1 hour, since precipitation was slow, before this second fraction was collected on the filter with the first. The precipitate was dissolved in water and used immediately, as described later.

The stock solutions of all of the salts tested were made to a concentration of 0.5 N. Potassium chloride, manganese chloride, ferrous chloride, and ferric chloride were used also in the additional

concentrations of 0.1 N and 0.01 N. Since there was always 5 cc. of water in the apparatus, the final concentration of the salt, there was 0.1 N for 0.5 N solutions and 0.02 and 0.002 N for 0.1 N and 0.01 N solutions used.

Oxidation was measured in centimeters of mercury rise by means of the simplified BUNZELL apparatus (8). The shaking machine was run at the rate of 106 complete excursions per minute. All experiments were run for 3 hours, readings being taken every 15 minutes, and a final reading the following morning. When bark was used, the mixtures in the apparatus contained 0.1 gm. of bark, 1 cc. of salt solution, and 4 cc. of 1 per cent pyrogallol solution or salt and pyrogallol with bark omitted, the second combination serving as a control on the first. Preliminary experiments had shown that during the time in which these experiments were run the autooxidation of the pyrogallol was usually not more than the equivalent of 0.15 cm. mercury rise. In the experiment with precipitated oxidase, the precipitate from 2 gm. of bark was dissolved in 20 cc. of water, and 2 cc. of the solution, containing the dissolved precipitate obtained from 0.2 gm. of bark, were put in each apparatus, together with the usual amount of pyrogallol and water. All tests were run in duplicate. Two controls were run with each experiment, one containing only water, the other bark (or oxidase solution), pyrogallol, and water, but without the addition of salts.

The figures for P_H given in table VII were obtained by means of the apparatus described by ROSE (28).

Discussion

The chlorides in general retard oxidase activity. The chlorides of potassium, sodium, and lithium depress markedly the oxidation of pyrogallol by bark (table I). Similar results were obtained with all the other chlorides tested, except ferrous chloride (table VI). Ferrous chloride in 0.1 N concentration with bark and pyrogallol showed 1.79 cm. mercury rise, and with pyrogallol alone 1.45 cm., compared with the control of pyrogallol and bark as 1.00 cm. Since ferrous chloride is readily oxidized when exposed to the air, it is quite probable that the oxygen absorption for the most part represents that absorbed in the oxidation of ferrous chloride.

Results

The results of the experiments are shown in tables I–VII and figs. 1–5.

TABLE I

EFFECT OF 0.1 N KCl, NaCl, AND LiCl ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 23.2–23.6° C.*

TIME OF READING	NO BARK			BARK			
	KCl	NaCl	LiCl	Check	KCl	NaCl	LiCl
May 21							
12.30.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.45.....	0.03	0.13	0.00	0.03	0.06	0.07	0.03
1.00.....	0.03	0.13	0.00	0.08	0.05	0.11	0.05
1.15.....	0.08	0.20	0.00	0.23	0.15	0.18	0.15
1.30.....	0.05	0.17	0.02	0.25	0.15	0.18	0.15
1.45.....	0.05	0.13	0.00	0.33	0.15	0.21	0.15
2.00.....	0.07	0.18	0.00	0.38	0.15	0.24	0.16
2.15.....	0.08	0.19	0.05	0.43	0.19	0.27	0.21
2.30.....	0.08	0.19	0.04	0.45	0.19	0.31	0.25
2.45.....	0.07	0.17	0.05	0.45	0.25	0.30	0.25
3.00.....	0.05	0.16	0.05	0.50	0.23	0.32	0.26
3.15.....	0.09	0.19	0.06	0.65	0.26	0.36	0.29
3.30.....	0.10	0.20	0.05	0.68	0.28	0.35	0.32
May 22							
8.40.....	0.00	0.00	0.00	1.25	0.80	0.74	0.77

* In tables I–V manometer readings in cm. of mercury corrected against an apparatus containing only water.

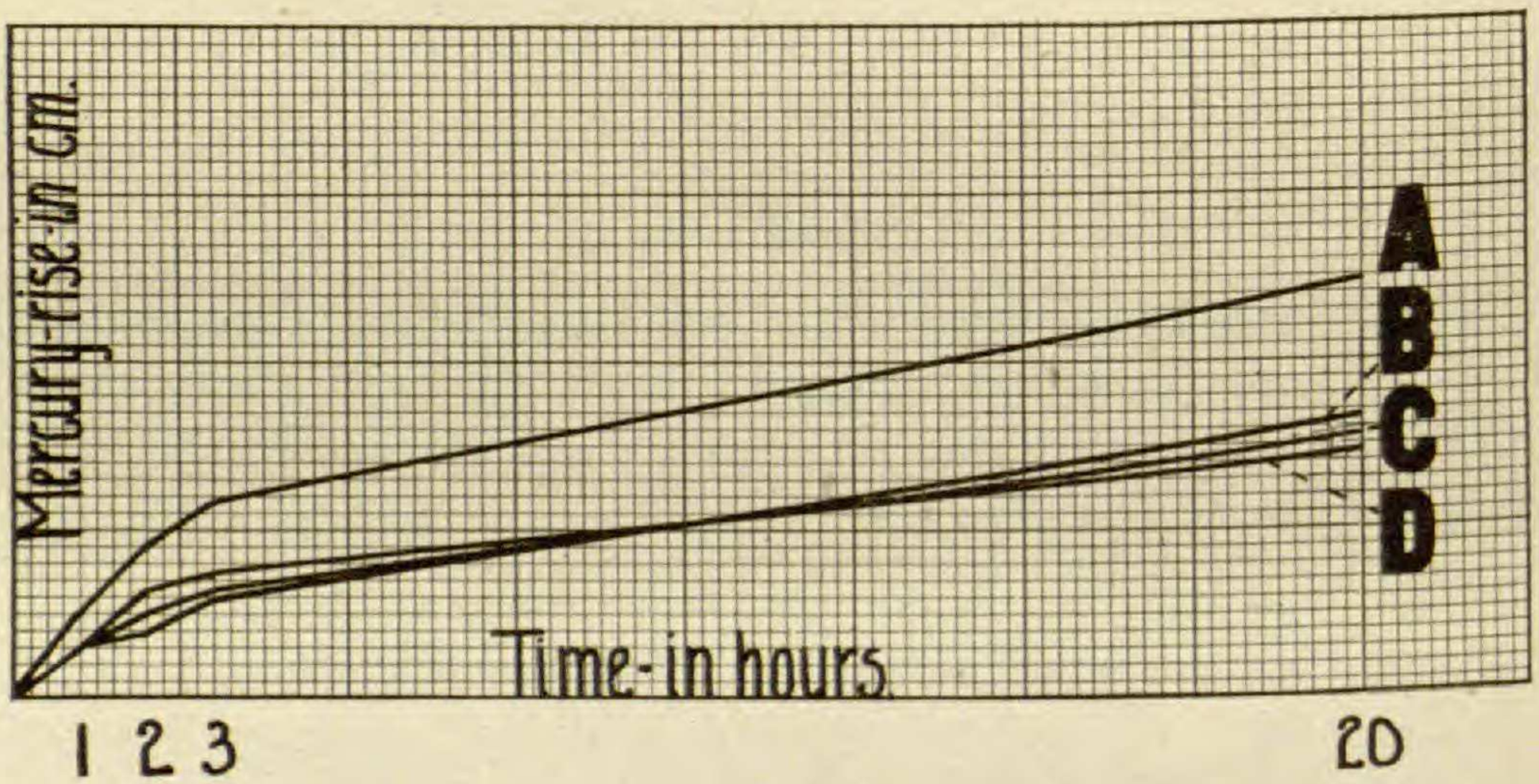

FIG. 1.—Effect of KCl, NaCl, and LiCl on oxidation of pyrogallol by powdered healthy apple bark: A, control (bark and pyrogallol); B, KCl+bark and pyrogallol; C, NaCl+bark and pyrogallol; D, LiCl+bark and pyrogallol.

TABLE II

EFFECT OF 0.10 N ALKALI CARBONATES ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 29.3–30.0° C.

TIME OF READING	NO BARK			BARK			
	K_2CO_3	Na_2CO_3	Li_2CO_3	Check	K_2CO_3	Na_2CO_3	Li_2CO_3
June 13							
1.30.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.45.....	0.55	0.50	0.54	0.14	0.08	0.10	0.13
2.00.....	1.35	1.13	1.19	0.19	0.48	0.47	0.48
2.15.....	1.75	1.55	1.64	0.24	0.75	0.85	0.82
2.30.....	2.10	1.78	1.92	0.34	0.93	1.02	1.02
2.45.....	2.40	2.05	2.12	0.44	1.16	1.27	1.23
3.00.....	2.50	2.15	2.24	0.49	1.33	1.42	1.33
3.15.....	2.60	2.28	2.34	0.54	1.44	1.52	1.50
3.30.....	2.90	2.41	2.55	0.63	1.63	1.76	1.74
3.45.....	3.00	2.47	2.57	0.68	1.79	1.86	1.85
4.00.....	3.05	2.54	2.64	0.74	1.87	1.97	1.93
4.15.....	3.08	2.60	2.69	0.79	1.93	2.02	1.99
4.30.....	3.17	2.68	2.75	0.86	2.08	2.19	2.22
June 14							
8.30.....	3.17	2.63	2.74	1.29	2.70	2.61	2.85

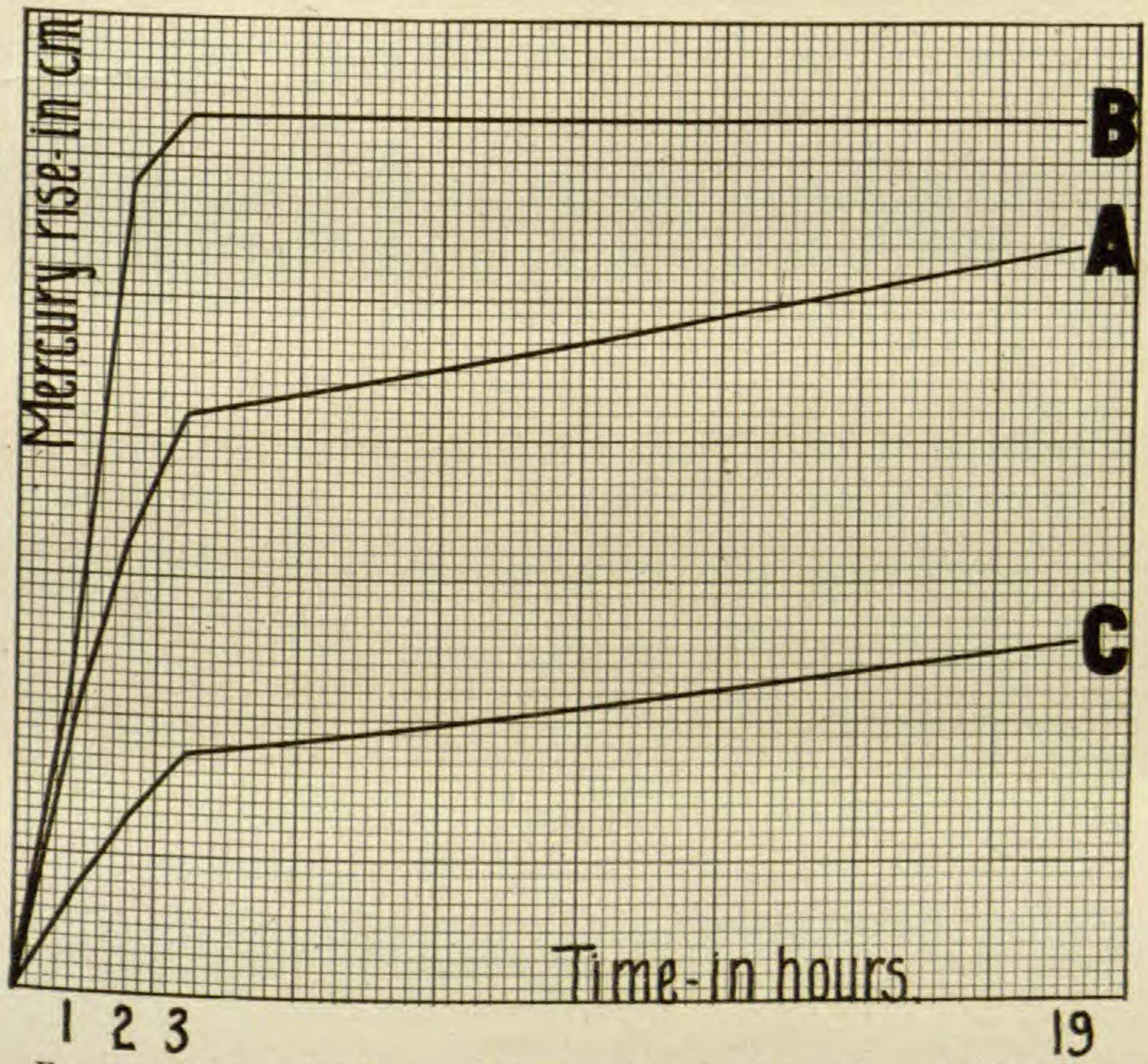

FIG. 2.—Effect of K_2CO_3 on oxidation of pyrogallol, with and without bark (healthy): A, K_2CO_3+bark and pyrogallol; B, K_2CO_3+pyrogallol; C, control (bark and pyrogallol).

TABLE III

EFFECT OF 0.10 N KCl AND K_2CO_3 ON OXIDATION OF PYROGALLOL BY POWDERED DISEASED APPLE BARK; TEMPERATURE 27.8–29.0° C.

TIME OF READING	NO BARK		BARK		
	K_2CO_3	KCl	Check	K_2CO_3	KCl
March 10					
10.00	0.00	0.00	0.00	0.00	0.00
10.15	0.68	−0.05	0.13	0.46	0.16
10.30	1.24	0.00	0.30	0.90	0.33
10.45	1.65	0.00	0.50	1.25	0.38
11.00	1.98	−0.08	0.65	1.50	0.48
11.15	2.25	−0.03	0.72	1.72	0.60
11.30	2.38	−0.03	0.85	1.93	0.69
11.45	2.52	0.00	0.99	2.09	0.82
12.00	2.65	−0.05	1.04	2.22	0.88
12.15	2.75	0.00	1.15	2.35	0.95
12.30	2.78	−0.05	1.18	2.39	0.95
12.45	2.85	−0.08	1.25	2.55	1.00
1.00	2.99	−0.05	1.38	2.65	1.10
March 11					
9.45	3.53	−0.10	2.20	3.73	1.73

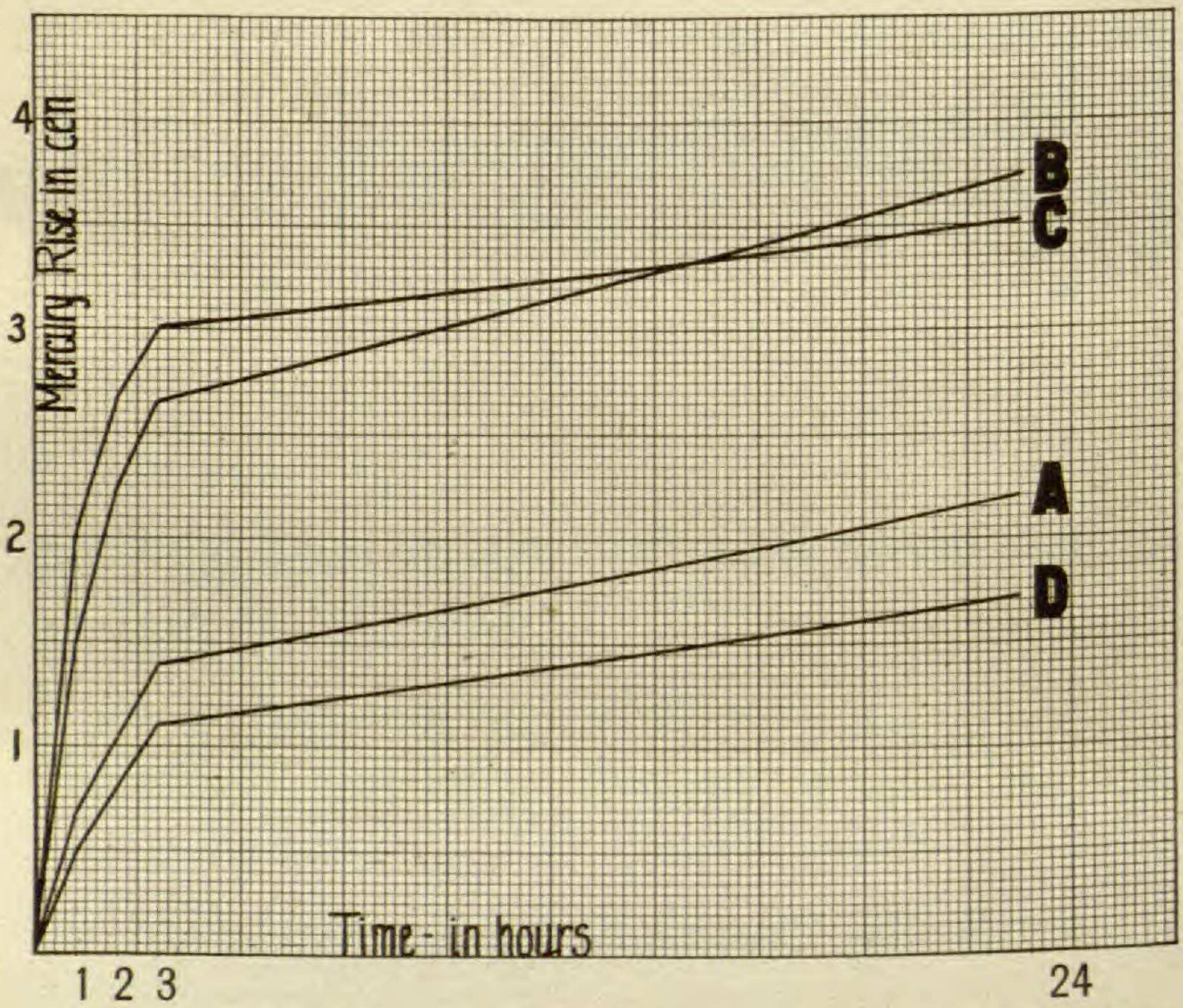

FIG. 3.—Effect of KCl and K_2CO_3 on oxidation of pyrogallol with and without bark (diseased): A, control (bark and pyrogallol); B, K_2CO_3+bark and pyrogallol; C, K_2CO_3+pyrogallol; D, KCl+bark and pyrogallol (KCl+pyrogallol gave no oxidation).

TABLE IV

EFFECT OF 0.10 N POTASSIUM TARTRATE, SODIUM OXALATE, AND $Ca(NO_3)_2$ ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 29.2–30.2° C.

TIME OF READING	NO BARK			BARK			
	Potassium tartrate	Sodium oxalate	$Ca(NO_3)_2$	Check	Potassium tartrate	Sodium oxalate	$Ca(NO_3)_2$
June 22							
1.30.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.45.....	0.11	0.08	0.18	0.10	0.14	0.20	0.08
2.00.....	.....	.....	.....	0.25	0.28	0.25	0.20
2.15.....	0.25	0.10	0.20	0.30	0.36	0.35	0.23
2.30.....	.....	.....	.....	0.38	0.48	0.46	0.30
2.45.....	0.35	0.19	0.20	0.43	0.58	0.55	0.35
3.00.....	.....	.....	.....	0.55	0.64	0.64	0.40
3.15.....	0.48	0.31	0.28	0.58	0.76	0.78	0.50
3.30.....	.....	.....	.....	0.70	0.98	0.80	0.55
3.45.....	.....	.....	.....	0.73	0.95	0.96	0.59
4.00.....	0.68	0.38	0.35	0.80	1.03	1.00	0.60
4.15.....	.....	.....	.....	0.90	1.13	1.10	0.73
4.30.....	0.78	0.35	0.38	0.90	1.15	1.13	0.70
June 23							
8.20.....	0.95	0.68	0.23	1.20	1.53	1.60	0.98

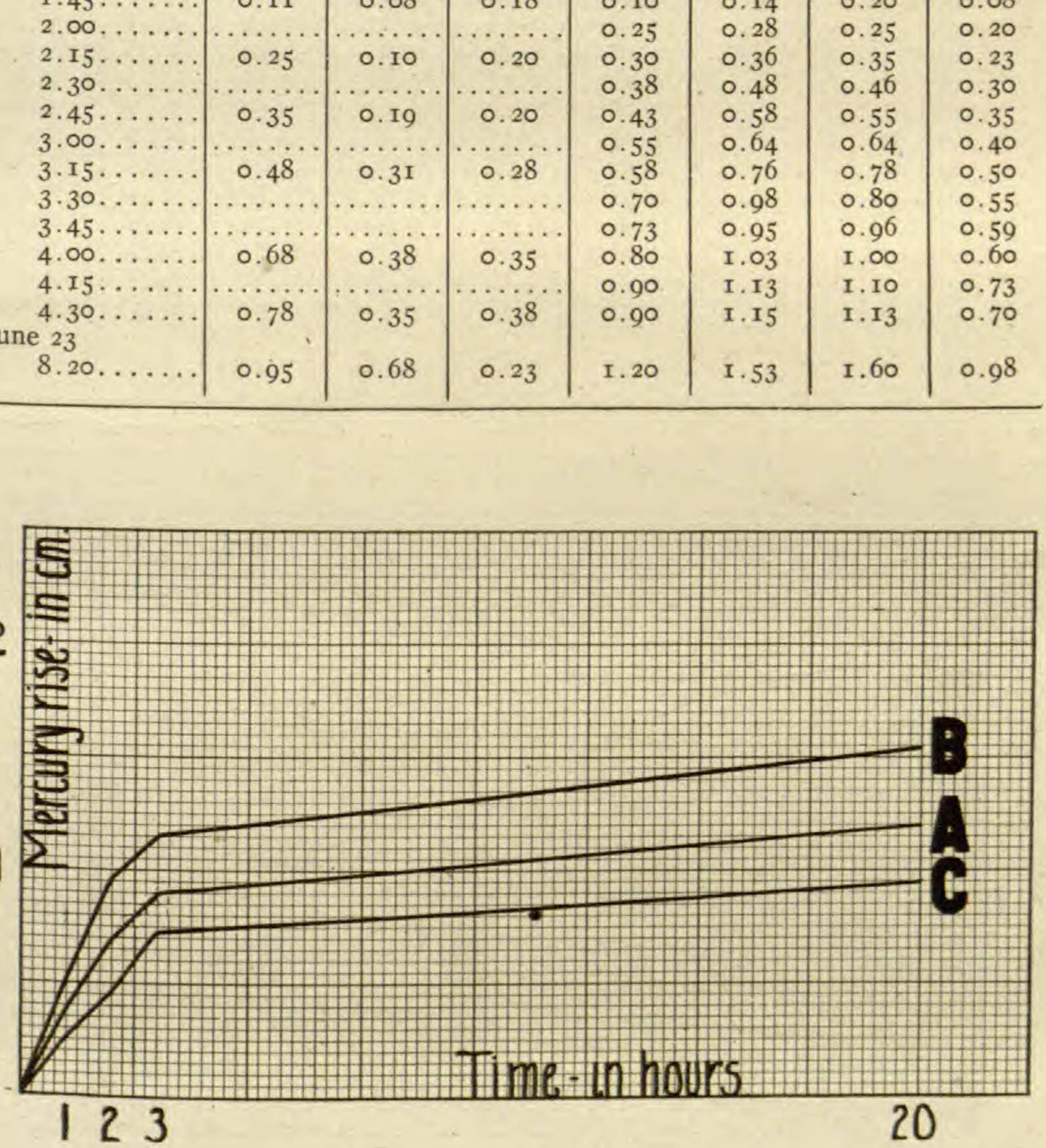

FIG. 4.—Effect of potassium tartrate on oxidation of pyrogallol with and without bark (healthy): A, control (bark and pyrogallol); B, potassium tartrate+bark and pyrogallol; C, potassium tartrate+pyrogallol.

TABLE V

EFFECT OF 0.10 N $MnCl_2$ AND K_2SO_4 ON OXIDATION OF PYROGALLOL BY PRECIPITATED OXIDASE FROM BOTH HEALTHY AND DISEASED APPLE BARK; TEMPERATURE 29.5–30.2° C.

TIME OF READING	HEALTHY			DISEASED		
	Check	$MnCl_2$	K_2SO_4	Check	$MnCl_2$	K_2SO_4
June 21						
1.45	0.00	0.00	0.00	0.00	0.00	0.00
2.00	0.07	0.08	0.11	0.17	0.15	0.15
2.15	0.08	0.10	0.21	0.37	0.29	0.30
2.30	0.08	0.13	0.23	0.42	0.21	0.33
2.45	0.08	0.13	0.27	0.48	0.25	0.43
3.00	0.08	0.10	0.25	0.50	0.23	0.48
3.15	0.15	0.11	0.28	0.56	0.24	0.54
3.30	0.15	0.08	0.30	0.65	0.26	0.58
3.45	0.18	0.09	0.35	0.70	0.29	0.63
4.00	0.20	0.08	0.34	0.79	0.31	0.69
4.15	0.20	0.08	0.37	0.87	0.34	0.78
4.30	0.23	0.09	0.38	0.88	0.35	0.78
4.45	0.28	0.18	0.43	0.98	0.40	0.93
June 22						
8.00	0.53	0.28	0.58	1.24	0.63	1.28

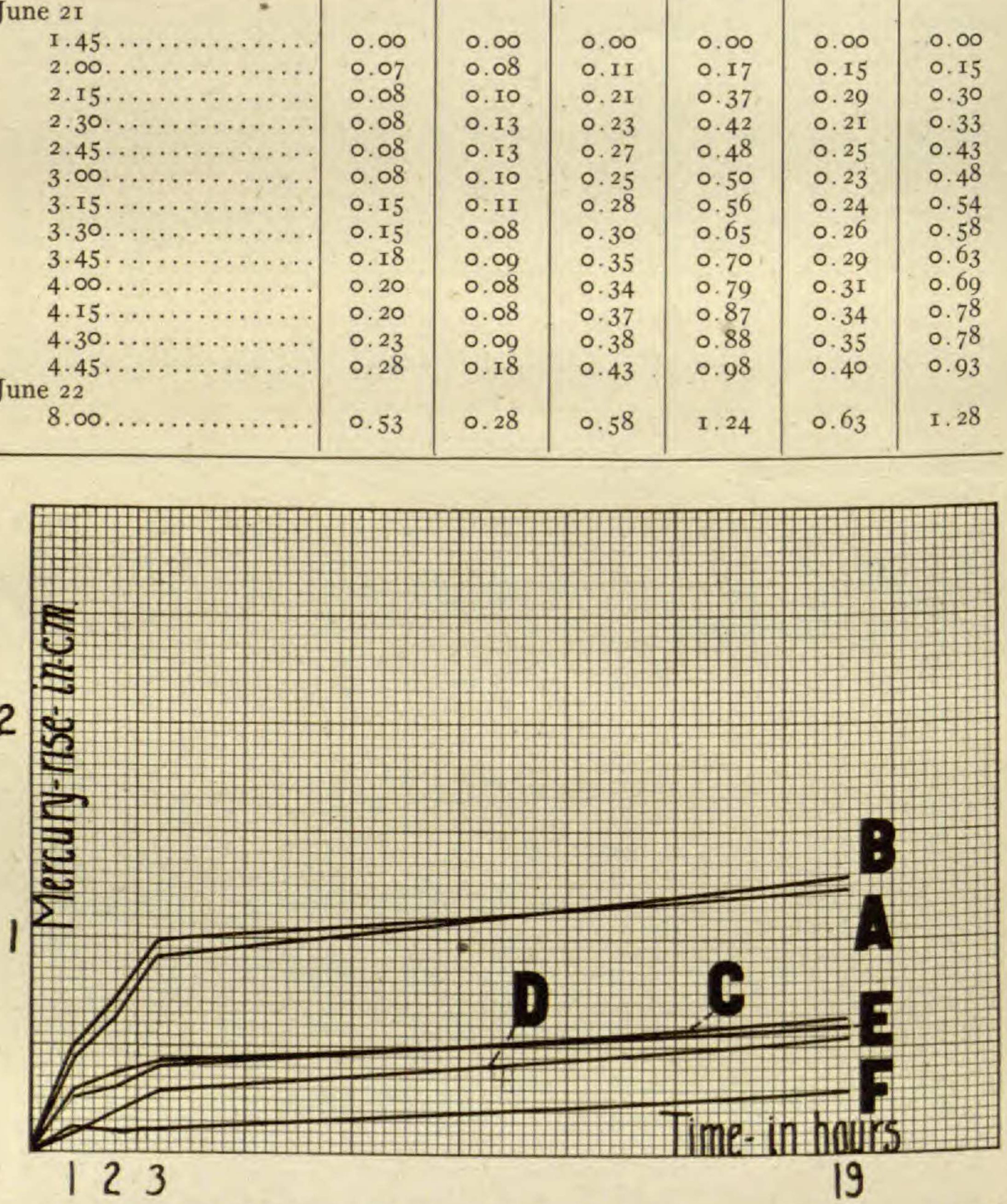

FIG. 5.—Effect of $MnCl_2$ and K_2SO_4 on the oxidation of pyrogallol on precipitated oxidase from both healthy and diseased bark: A (diseased), K_2SO_4+bark and pyrogallol; B (diseased), control (bark and pyrogallol); C (diseased), $MnCl_2$+bark and pyrogallol; D (healthy), K_2SO_4+bark and pyrogallol; E (healthy), control (bark and pyrogallol); F (healthy), $MCnl_2$+bark and pyrogallol.

TABLE VI

SUMMARY OF EFFECT OF ALL SALTS TESTED ON OXIDATION OF PYROGALLOL WITH AND WITHOUT BARK; ALL FIGURES CALCULATED TO BASIS OF 1 CM. MERCURY RISE FOR CHECK.

Salt		Check	Cl		SO_4		NO_3		CO_3		PO_4		H_2PO_4		Tartrate		Oxalate		Acetate		Citrate	
			With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
K	0.1 N...	1.00	0.63	0.00	1.07	0.08	0.99	0.07	2.09	2.46	1.85	1.90	0.96	0.16	1.27	0.76	1.16	.....	.....	.....	.....	.....
K	0.02 N..	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.04	.....	.....	.....	.....	.....	.....	.....	.....	.....
K	0.002 N.	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.07	.....	.....	.....	.....	.....	.....	.....	.....	.....
Na	0.1 N...	1.00	0.59	0.00	1.06	0.06	0.98	0.09	2.02	2.11	.....	.....	.....	.....	1.14	0.26	1.33	0.55	1.55	1.84	2.06	2.41
Li	0.1 N...	1.00	0.61	0.00	1.05	0.10	....	.....	2.20	2.19	.....	.....	.....	.....	.....	.....	.....	.....	1.37	1.28	1.92	2.09
Cs	0.1 N...	1.00	0.66	0.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
NH_4	0.1 N...	1.00	0.69	0.17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ca	0.1 N...	1.00	0.57	0.15	.....	.....	0.80	0.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mg	0.1 N...	1.00	.....	.....	.....	.....	0.94	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	0.1 N...	1.00	0.54	0.10	1.04	0.02	0.76	0.04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	0.02 N..	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	0.002 N.	1.00	1.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ba	0.1 N...	1.00	.....	.....	.....	.....	0.84	0.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe'''	0.1 N...	1.00	1.79	1.45	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe'''	0.02 N..	1.00	1.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe'''	0.002 N.	1.00	1.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''	0.1 N...	1.00	0.24	0.05	.....	.....	0.31	0.24	.....	.....	.....	.....	.....	.....	.....	.....	0.45	0.06	.....	.....	.....	.....
Fe''	0.02 N..	1.00	0.55	.....	.....	.....	0.55	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''	0.002 N.	1.00	0.86	.....	.....	.....	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	0.1 N...	1.00	0.50	.....	1.03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
K	0.1 N...	1.00	0.52	.....	1.09	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE VII

RELATION OF OXIDATION TO INITIAL P_H OF MIXTURES*

SALT	CHECK		Cl		SO_4		NO_3		CO_3		H_2PO_4		TARTRATE		OXALATE		ACETATE		CITRATE	
	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H
K	1.00	5.15	0.63	5.19	1.07	5.13	0.99	5.14	....	.....	0.96	4.47	1.27	6.00	1.16	5.77	.....	5.72	.....	5.65
Na	.....	.....	0.59	5.17	.....	.....	.....	.....	.....	.....	.....	.....	1.14	.....	1.33	.....	1.55	6.43	2.06	6.02
Li	.....	.....	.....	.....	1.05	5.06	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.37	5.97	1.92	6.19
NH_4	.....	.....	0.69	4.85	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ca	.....	.....	0.57	4.79	.....	.....	0.80	4.74	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mg	.....	.....	.....	.....	.....	.....	0.94	4.62	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	.....	.....	0.54	4.48	1.04	4.50	0.76	4.43	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ba	.....	.....	.....	.....	.....	.....	0.84	4.78	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''	.....	.....	0.24	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Concentration of salts 0.10 N.

This view is substantiated by the fact that when the concentration of ferrous chloride is reduced, oxygen absorption is reduced proportionally (table VI). If we subtract 1.45 cm. (mercury rise for pyrogallol and ferrous chloride) from 1.79 cm. (mercury rise for bark, pyrogallol, and ferrous chloride), we have 0.34 cm. for the oxidase activity of the bark in the presence of the ferrous chloride as compared with 1.00 cm. for the oxidase activity of bark and pyrogallol in the absence of ferrous chloride. Apparently ferrous chloride retards oxidase activity just as the other chlorides do, and the increased absorption of oxygen in the presence of ferrous chloride is due to the action of ferrous chloride itself in absorbing oxygen. Oxidation is increased by 0.002 N manganese chloride. This is in accord with the results of BERTRAND (5) and others. In a concentration of 0.1 N it inhibits oxidation just as do the other chlorides.

The use of precipitated oxidase shows that chlorides have a depressing effect on oxidation, even under conditions which eliminate many of the substances present in the bark powder. No investigation has been made of the effect of these substances on the reaction, but they probably complicate it.

The results with the chlorides are in accord with the work of EWART, who found that dilute solutions of potassium chloride and sodium chloride prevent the browning of slices of apples. EWART'S further conclusion, however, that the chlorides act as sensitizers to oxidation, or ALSBERG'S idea that chlorine plays an important part in the bluing of guaiacum by the chlorides of metals, are scarcely borne out by our observations that chlorides in general depress oxidase activity. It should be noted, however, that the results of those investigators were based upon color reactions, while ours were based upon oxygen absorption.

It is interesting to note that the chlorides which retard the combustion of tobacco at high temperatures have a similar effect in depressing oxidase activity. KRAYBILL (21) has suggested that the chlorides may have a negative catalytic action in the case of the combustion of tobacco. It would be interesting to know how the chlorides affect other oxidation processes.

The depressing effect of chlorides on oxidase activity is in contrast with their action on other enzymatic processes. Thus NASSE (25), KÜBEL (22), COLE (10), WOHLGEMUTH (31), LISBONNE (23), HAWKINS (18), and others have found that chlorides increase the diastatic power of various preparations of diastase. NASSE, however, found that under certain conditions sodium chloride retarded diastatic activity, and later HAWKINS showed that sodium chloride and potassium chloride in certain dilute concentrations (M/128–M/512) retard diastatic activity. It would have been better if the effect of the chlorides upon oxidase activity had been determined in a greater number of concentrations, and it will be well in the future to do so in studying this problem. The effect of salts upon lipase activity is also of interest in this connection. LOEVENHART and PEIRCE (24), GERBER (14), TERROINE (30), HAMSIK (16), FALK (12), and others found that the chlorides of various alkalies and alkaline earths retard lipase activity. TERROINE found that the concentration of the salts which he studied determined the nature of their influence. BUCHNER, BUCHNER, and HAHN (7) found that the chlorides of sodium, calcium, barium, and ammonium inhibit the fermentation of cane sugar or glucose in the presence of pressed yeast.

The results presented in table VI do not show any marked difference in the behavior of the different chlorides tested. The cations, judging from the limited data available, apparently have little or no effect; or at least their chlorides all behave very much in the same manner. In this respect the alkali salts are different in their effect upon the fire-holding capacity of tobacco, for here the salts of caesium, rubidium, and potassium in general are much more favorable to combustion than the corresponding salts of sodium or lithium. A similar contrasting behavior of different cations of chlorides was noted by HARDEN (17), who found that potassium chloride and ammonium chloride cause a definite degree of fermentation in inactivated yeast, while sodium chloride has no effect. He says: "A specific difference in relation to alcoholic fermentation exists between the ions of sodium on the one hand and of potassium and ammonium on the other hand." SCHREINER and SULLIVAN (29) found that potassium salts retard oxidation by the roots of plants.

The effect of the chlorides of the alkalies in retarding oxidase activity suggests a possible practical application in preventing the browning of fruits and vegetables during their preparation for canning, preserving, or drying.

The sulphates apparently increase oxidation slightly in all cases, but the readings are not sufficiently large to be of any positive significance.

The nitrates of potassium, sodium, and magnesium have no marked effect on oxidation, while the nitrates of barium, calcium, manganese, and iron (ferric) decrease it. These results are similar to the effect upon respiration as found by ZALESKI and REINHARD (33). FERNBACH and LANZENBERG (13) and KAYSER (20) find that nitrates increase alcoholic fermentation, but, as they point out, the effect may be to increase multiplication of the yeast cells rather than to affect enzymatic action.

In tables II and III and figs. 2 and 3 are shown the oxidation of pyrogallol by bark alone, by bark and carbonate, and by carbonate alone. From these it is seen that in the last two cases oxidation is considerably greater than that by the bark alone. It is also seen that during the first 3 hours oxidation by carbonate is greater than that by carbonate and bark, but that after the experiment has stood overnight oxidation by healthy bark and carbonate approaches that by carbonate alone, and oxidation by diseased bark and carbonate exceeds it.

The most obvious explanation of this fact, although possibly not the true one, is that oxidation by a carbonate is a strictly chemical reaction, catalyzed only by hydroxyl ions, which soon comes to a definite end, while oxidation by carbonate and bark is a reaction catalyzed by both "oxidase" and hydroxyl ions, in which the presence of the hydroxyl ions increases the effectiveness of the "oxidase," which is slow in reaching an end-point.

Table VI shows that tripotassium phosphate increases oxidation of pyrogallol very markedly, both with and without bark. Although no P_H values for this mixture are available, we know the salt is alkaline in reaction, and this effect complicates the matter. With potassium dihydrogen phosphate at 0.10 N concentration a decrease is evident, and at 0.02 N and 0.002 N concentrations a

slight increase in oxidation occurs. The higher hydrogen ion concentration is probably the cause of the slight depression in oxidation of the 0.10 N strength of the salt. The slight increase in oxidation of the lower concentrations suggests that phosphates may increase oxidase activity, but the limited data are inconclusive. It is interesting to note that IWANOFF (19) found that phosphates raise the amount of respiration in living wheat seedlings. ZALESKI and REINHARD (33) found that disodium phosphate increases the output of carbon dioxide from dried ground seeds, and that the monobasic phosphate decreases it because of the acid reaction. These authors also quote from the work of a student, Miss SCHKLOUSKY, who showed that phosphates increase the action of peroxidases, and from work of another student, Miss ROSENBERG, who showed that phosphates stimulate the catalase activity of different seeds.

In the case of salts of organic acids and the carbonates, all more alkaline than any of the inorganic salts (table VI), oxidation is greater at all stages of the experiment when bark is used than when it is not. Examples of this are shown in table IV. The effect of the salt is not merely additive, however, either here or in the case of the carbonates, as is shown by the following:

OXIDATION OF PYROGALLOL BY BARK AND SALT

	Tested separately (cm. of mercury rise)	Tested together (cm. of mercury rise)
K_2CO_3	4.46	2.70
K tartrate	2.15	1.53
Na oxalate	1.88	1.60

Evidently when bark and salt are combined, there is some factor at work which brings about a slower rate of oxidation than might be expected. What this factor may be we have no means of knowing as yet. Possibly it is the partial neutralization of the hydroxyl ions of the salt by the acid of the bark.

The question why salts vary so widely in the effect they have on oxidation is not easily answered. If we consider only the results with 0.1 N solutions, it seems clear, in the case of the carbonates, potassium dihydrogen phosphate, and the salts of organic acids here reported, that increased oxidation in their presence is due to the excess of hydroxyl ions they furnish; that is, by the

reaction (P_H) their solutions establish when mixed with bark and pyrogallol (table VII). The reaction established by the chlorides, however, can hardly be responsible for the decrease in oxidation they bring about, since sulphates, giving about the same reaction, cause a small increase in oxidation. For example, a mixture of potassium chloride, bark, and pyrogallol has a P_H of 5.19 and gives only 63 per cent as much oxidation as the control. A similar mixture containing potassium sulphate has a P_H of 5.13 and gives 7 per cent more oxidation than the control. The corresponding figures for manganese are: manganese chloride mixture, $P_H = 4.50$, oxidation = 104 per cent of the control.

The situation for nitrates shows several irregularities. Potassium nitrate giving a P_H of 5.14 has practically no effect on oxidation. Magnesium nitrate is also without effect, but gives a P_H of 4.62. The nitrates of calcium, barium, and manganese inhibit oxidation, but manganese gives a lower P_H and the other two a higher one than that given by magnesium nitrate.

The results presented justify the conclusion that when 0.1 N solutions of the salts are used, other ions than hydrogen and hydroxyl play an important part in controlling oxidation. When hydrogen or hydroxyl ions are neutralized in making oxidase activity determinations, therefore, it is important to take into consideration the possible effect of the salts formed thereby. This must be considered as merely preliminary to the real investigations of the relation of specific ions to the oxidation processes in plants and animals. The effect of iron and manganese salts has long been known, but more work is necessary, both with these and with the more commonly occurring chlorides, sulphates, and nitrates of other cations.

Summary

1. One-tenth normal solutions of all of the chlorides tested (potassium, sodium, lithium, caesium, ammonium, calcium, manganese, ferric) decreased oxidation of pyrogallol by apple bark powder.

2. Oxidation was increased very slightly by 0.10 N solutions of all the sulphates tested.

3. Potassium, sodium, and magnesium nitrates (0.10 N) had practically no effect on oxidation, while nitrates of calcium, barium, manganese, and iron (ferric) decreased it.

4. Potassium chloride (0.02 N and 0.002 N) had no effect on oxidation, while manganese chloride in these concentrations increased it.

5. Tartrates, oxalates, citrates, acetates, and carbonates increased oxidation. Marked increase in oxidation in these cases seems to be due, in part at least, to the low acidity of the mixtures of bark, pyrogallol, and salt.

6. Marked decrease in oxidation is not necessarily accompanied by high acidity of the mixtures.

7. Ions other than the hydrogen and hydroxyl may be important in regulating oxidase activity.

8. In neutralizing hydrogen or hydroxyl ions, it is important to take into consideration, in the study of oxidase activity, the possible effect of the salts formed thereby.

9. The chlorides which retard the combustion of tobacco at high temperatures also retard the oxidase action at low temperatures.

10. The effect of the alkali chlorides upon oxidase activity suggests a practical application in preventing the browning of fruits and vegetables during their preparation for canning, preserving, or drying.

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